

**Evaluation of Biochemical and Phytochemical Parameters of *Solanum
nigrum* L. and *Leucas aspera* (willd.) Linn.**

Karthika, A.

(Reg No: 14PBO003)

Thesis submitted to

**Avinashilingam Institute for Home Science and Higher Education for
Women**

Coimbatore- 641043

In Partial Fulfillment of the Requirements for the

Master's Science in Botany

April 2016

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29/4/16

Signature of the

Head of the Department


29/4/16

Signature of

the Supervisor

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CONTENT

CHAPTER NO	TITLE	PAGE NO
	LIST OF PLATES	
	LIST OF TABLES	
	LIST OF FIGURES	
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	7
III	MATERIALS AND METHODS	14
IV	RESULTS AND DISCUSSION	28
V	SUMMARY AND CONCLUSION	41
	REFERENCES	42

LIST OF PLATES

PLATE NO	TITLE	PAGE NO
1	Morphology of the plant - <i>Solanum nigrum</i>	16
2	Morphology of the plant - <i>Leucas aspera</i>	19
3	Leaf powder of the <i>Solanum nigrum</i>	26
4	Leaf powder of the <i>Leucas aspera</i>	26

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
1	Chlorophyll 'a', Chlorophyll 'b' and Total Chlorophyll	
	Contents of the two medicinal plants	32
2	Protein content of the medicinal plants	34
3	Carbohydrate content of the medicinal plants	36
4.	Preliminary phytochemical analysis of <i>Solanum nigrum</i>	38
5.	Preliminary phytochemical analysis of <i>Leucas aspera</i>	40

LIST OF FIGURES

**FIGURE
NO**

TITLE

**PAGE
NO**

1	Chlorophyll 'a,' Chlorophyll 'b' and Total Chlorophyll contents of the two medicinal plants	31
2	Protein content of the two medicinal plants	34
3	Carbohydrate content of the two medicinal plants	36

CHAPTER-I

INTRODUCTION

The World Health Organization (WHO) approximated that around 80% of earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components (Bruneton, 2001). A few generally used herbs had been identified by the National Cancer Institute (NCI) as possessing cancer preventive properties; these herbs include members of different families (Caragay, 1992). The people of rural area are mainly depending on the traditional medicine for curing their ailments because of the non-availability of modern medicines and hospitals. In developing countries, 80 % of the population still use traditional folk medicines obtained from natural resources. In India, with more than 75 % of the population residing in rural areas close to the natural resources, rich traditions of utilizing medicinal plants have existed among indigenous people for age.

Plants have healing powers on man and animals. Medicine from plant sources have been in use in Homeopathy, Ayurveda, Allopathy and in traditional medicine since time immemorial. Medicinal plants play a significant role among the traditional and modern systems. Their use has been multiplied through various researches and application due to a number of side effects from use of synthetic drugs, antibiotics and high cost. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least, 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects.

Increasing use of herbs for healthcare and the herbal medicine boom in recent years has imposed a great threat to the conservation of natural resources and endangered plant species. The high demand of medicinal plants in both domestic and international markets has resulted in the over harvesting of certain limited natural resources, thus depriving the forest resources at an unprecedented rate and causing serious ecological damage. Important

components of forest ecosystem are trees, which provide food, fuel, construction, industrial and medicinal products. In addition, they are recognized as the critical elements in maintaining stability of the world atmosphere (Harry and Thorpe, 1994).

The medicinal plants are useful for healing, as well as, for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Abdul wadood, 2013). The use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use of traditional medicine, provided they are proven to be efficacious and safe.

Phytochemicals are active components found in plants that act against diseases. They are non-nutritive compounds that contribute to flavour colour. Many phytochemicals have antimicrobial activity and reduce the risk of many diseases. Plant derived products has been used for their phytochemicals with various bioactivities, including, antimicrobial, antioxidant, anti-inflammatory and anticancer activities (Barathi, 2014). Alkaloids, Flavonoids, Phenolic Compounds, Tannins and Saponins are responsible for the function of antimicrobial activity. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas.

Phytochemical investigations carried out during the 1970's and 1980's have discovered a number of alkaloids and other pharmacologically active substances (Ajitkumar *et al.*, 2015). The chemical constituents of plant play an important role in modern medicine after profiling against different biological activities. About three quarters of population of the world are estimated to be dependent mainly on plants and plant extracts for the care of their health. Medicinal plants possess potent medicinal value that is due to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body (Muhammad Zubair *et al.*, 2011).

Although, herbal medicines have long been used effectively in treating diseases in Asian communities and throughout the world, the mechanism of most of the herbals used has not been defined. Many traditional plants treatment for their beneficial effects are anecdotal. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can

counter the high cost and poor availability of the current medicines. India is well known for its herbal wealth. However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed.

SOLANUM NIGRUM

Solanum nigrum commonly known as “Black night shade” belongs to Solanaceae family. It is called as Manathakkali in Tamil. This family consists of 90 genera and approximately 2000-3000 species. In this family, *Solanum* constitutes the largest and the most complex genus and it consists of more than 1500 species, many of which are also economically important throughout their cosmopolitan distribution. The generic name *Solanum* is considered to be derived from the Latin “Solamen” to search to the reticent or lenitive effects associated with many species. It is commonly known as Makoi in Hindi.

Solanum nigrum is found mainly around waste land, old fields, ditches and roadside, fence rows, or edges of woods and cultivated lands (Atunu and Ajayi, 2011). *S. nigrum* has been extensively used in traditional medicine for various ailments such as pain, inflammation and fever (Muhammad Zubair *et al.*, 2011). It shows medicinal properties like antimicrobial, antioxidant, cytotoxic properties, antiulcerogenic and hepatoprotective activity. *S. nigrum* has been used in treating liver diseases. The plant also possesses antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic and antipyretic properties (Ayesha *et al.*, 2010). The berries have been used in the treatment of stomach ulcers and in folk medicine, the juices from leaves are used to treat tonsillitis (Singh *et al.*, 2011). It is an African paediatric plant utilized for a few ailments that are responsible for infant mortality especially feverish convulsions, eye diseases, hydrophobia and chronic skin ailments. It is a potential herbal alternative that acts as an anti-cancer agent (Jain *et al.*, 2011).

Evaluation of phytochemical constituents of the leaves, flowers and fruits of three valuable medicinal species of the genus *Solanum* has been carried out by (Gnana Sundari *et al.*, 2013). Solamargine is the main constituent actually responsible for its anticancer activity (Hu, 1999).

This herbal plant is used to treat asthma, blood vomiting, reducing blood glucose level and bilious matter phlegmatic rheumatism and ulcer (Ramya *et al.*, 2012). The leaf extracts of *S. nigrum* has pharmacological application (Bhavani *et al.*, 2015).

S. nigrum is generally used in many traditional systems of medicine worldwide for disparate ailments, but has not garnered attention for modern therapeutic use (Ramya Jain *et*

al., 2011). It is a medicinal plant commonly used in Africa in ethnomedicine for use in a wide range of disorders including epilepsy. Epilepsy is a brain disorder in which clusters of nerve cells, or neurons in the brain consistently signal abnormally. Neurons normally generate electrochemical impulses that act on other neurons, glands and muscles to produce human thoughts, feelings and actions. In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensations, emotions and behaviour, or sometimes convulsions, muscle spasms and loss of consciousness (Hoang Le Son, 2014).

In addition to many local uses in Africa, certain species of *Solanum* are potentially globally important for agriculture, human health, plant breeding and biotechnology. The leaves and berries are a potential source of colouring plant extracts, inks and dyes, and they are rich in proteins, fibres, vitamins and amino acids. The therapeutic properties of the opium poppy (active principle morphine) were known in Ancient Egypt and those of the *Solanaceae* plants in ancient Greece (active principles atropine and hyoscine).

LEUCAS ASPERA

Leucas aspera (Willd) Linn. is a medicinal herb that belongs to the family Lamiaceae (Labiatae). It is popular as “Thumbai” throughout the Indian sub-continent. Among several other colloquial names, “Dhronpushpi” is common in North India. This is distributed throughout India from Himalayas down. It grows as a weed on waste lands and road sides all over India and is widely distributed in and around forest areas of East Godavari District, Andhra Pradesh, India. It is also found in Africa, Nepal, Pakistan, Srilanka, Malaysia, Myanmar and Thailand.

L. aspera possesses immense medicinal properties such as antipyretic, larvicidal, insecticidal, anti-inflammatory, antimicrobial and antioxidant. Leaves and stems are used as oral antihyperglycemic (Parameshwar *et al.*, 2010). The hepatoprotective effect of *Leucas aspera* in simvastatin induced hepatotoxicity (Mohd Abdul Irfan *et al.*, 2012) has been studied. *Leucas aspera* (darkolos or dandokolos in Bangladesh) is a common aromatic herb and grows abundantly in Bangladesh and also in the wide area of South Asia. In some forms of traditional medicine, the plant's flowers, seeds, roots, berries, bark or leaves can be inhaled to help treat nasal congestion, coughing, cold, headache and fever (Riazunnisa *et al.*, 2013).

The leaf extract of *Leucas* contains chemical constituents such as sterols, fatty acids, lactones, long chain compounds, aliphatic ketols and phenols (Pradhan *et al.*, 1990, Misra *et al.*, 1995). Leaf juice is used by villagers to arrest bleeding from cuts and bruises in animals.

In West Africa, the plant ash is used to withstand mosquitoes (Muhammad *et al.*, 2012). In Nigeria, the decoction of the leaves is used for the treatment of skin rashes, epilepsy and convulsions. The phytochemical components of the aqueous leaf extracts of *Leucas martinicensis* have been analysed by Eze *et al.*, (2013). Leaves are consumed as vegetable throughout the state of Assam and provide food security during times of drought and poor harvest.

Leucas aspera is a traditional medicine with multiple health common benefits. However, the medicinal values of the plant pertaining to wound has not yet been reported. Therefore, the aim of treating a scar is either to shorten the time required for healing or to accelerate the wound healing process.

Parkinson's disease (PD) is one of the major neurodegenerative disorders that affect the nerve cells in the part of the brain controlling muscle movement. People with Parkinson's disease often experience trembling, muscle rigidity, difficult walking, problems with balance and slowed movements. These symptoms usually develop after the age of 60.

Leucas aspera is a plant which has been used in Indian traditional system of medicine in the treatment of separate skin diseases and also as insecticides. The leaves are useful in the treatment of chronic rheumatism and skin eruptions. Skin is the first line of defense mechanisms of the body. Skin is the largest and well known organ of the body, which comprises 15% of our body weight. Skin harbours large number of normal flora and has good resistant mechanism. During unfavourable condition, pathogen and opportunistic pathogen will cause severe infection in the skin. Wound is defined simply as the disruption of cellular and anatomic continuity of a tissue (Bennet, 1988). Wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regain of strength of injured tissue.

There is a growing focus on the importance of medicinal plants and traditional health systems in solving the health care problems of the world. Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, TB and pain. Recently introduced drugs from plant origin include Taxol, podophyllotoxin, camptothecin, arteether, galantamine, nitisinone, and tiotropium (Shih, J. H., Charng, 2011).

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several medicines have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. As such, herbal remedies have been used to cure a variety of disorders or conditions such as diabetes, cardiovascular problems, weight control, dermal infirmities, sexual malfunction and of course cancer. *Leucas aspera* is a well-known plant that produces chemicals to protect humans against these diseases.

The plant is used traditionally in rheumatism, gum troubles, toothache, diarrhea and gonorrhoea and also as purgative (Ghani, 2003). However, to our knowledge, there is no scientific report on the verification of the use of the plant in the treatment of rheumatism. Inflammation has been reported to be the main pathological characteristic of rheumatism such as rheumatoid arthritis (Zhang *et al.*, 2008; Rewatkar *et al.*, 2010).

The rich traditional knowledge base countries like India and China in medicinal plants and health care have led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programme in the pursuit of discovering novel drugs. India has a varietal emporium of medicinal plants and it is one of the richest countries in the world in terms of genetic resources of medicinal plants (Krishnaraju, 2005).

The secondary metabolites in different plants make up a vast repository of compounds with a wide range of biological activities (Venkatachalam and Jebasan, 2010). The alkaloids are heterocyclic, nitrogen containing compounds, usually with potent activity and bitter taste. They are of limited distribution in the plant kingdom. The separate groups have diverse clinical properties. Most saponins “soap forming compound” occur as glycosides. It has also been used to prevent and to cure diarrhoea. For the treatment of fevers, the fresh leaves are rubbed on or the whole plant made into an infusion and used as a wash or steam fumigation (Fowler, 2006).

AIM AND OBJECTIVES

- To analyse the biochemical parameters in the freshly collected leaf sample of *Solanum nigrum* and *Leucas aspera*.
- Screening for the presence of phytochemical constituents in the two test plants.

CHAPTER – II

REVIEW OF LITERATURE

It is difficult to sort out the literature according to various taxa grouped *Solanum nigrum* complex because of the tendency of researchers to call them all as *Solanum nigrum* or occasionally as different species of genus *Solanum* or sometimes as variety of *Solanum nigrum*.

Chiej *et al.* (1984) has observed the use of *Solanum nigrum* in the manufacture of locally analgesic ointments. The plant has also been used as insecticide and larvicide (Singh *et al.*, 2001).

Nightshade is extensively used for the treatment of burns and ulcers by Arabs, as an antiseptic, anti-inflammatory, antidyenteric were also recorded for *Solanum nigrum* L. (Ceiej, 1984; Heiser 1969; Vogel 1990).

Wink *et al.* (1990) reported that plants produce an array of natural products, the so called secondary metabolite, which play a variety of roles such as defence molecules against attacks by animals and microorganisms. These substances are also important to man as a source of pharmaceuticals, fragrance, agrochemicals and food additives. Plants still remain as the major source of many vital medicinal compounds. Kloucek *et al.* (2005) reported that plants are used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders.

It has been reported to have about 30 morphologically distinct taxa and is named as *Solanum nigrum* complex (Schilling & Andersen, 1990).

Kannappa Reddy *et al.* (1992) studied the anti-ulcer activity of *Leucas aspera* Spreng. The alcoholic extract of *Leucas aspera* (ALA) was investigated for its anti-ulcer effect by two experimental models.

The use of *Leucas* species for the treatment of separate ailments like cholera (Reddy *et al.*, 1993), malaria (Valsaraj *et al.*, 1997), syphilis (Rahman *et al.*, 2007), diarrhoea and dysentery (Sharma *et al.*, 1992) have been studied. The leaves are also taken personally for pain during pregnancy (Qamaruddin *et al.*, 2002). The infusion is treated ophthalmically for conjunctivitis (Qamaruddin *et al.*, 2002) and for corneal disease (Dhar *et al.*, 1968)

The genus *Solanum nigrum* contains about 1400 species of plants distributed throughout the temperate and tropical regions of the world (Eltayeb *et al.*, 1997). The species *Solanum nigrum* L. (black nightshade) grows abundantly in the wild in open fields in the north eastern region of Asia and it is known to have medicinal importance (Roddick, 1991). Plants of *S. nigrum* have been traditionally used in the Orient as herbal medicine.

Solanum is one of the largest and hyper diverse genes of the family Solanaceae for its usefulness to mankind. The members of genus are being used for medicinal purpose from the ancient time.

In India, *Solanum nigrum* is used as hepatoprotective agent, and in Mexico, the fruits of this plant are used as a tonic for treating various nerve disorders (Perez *et al.*, 1998). A previous study revealed that ethanol extracts of *Solanum nigrum* suppressed the oxidant-mediated degradation of calf thymus DNA (Sultana *et al.*, 1995). Other studies showed that the plant exerted cytoprotection against gentamicin toxicity on Vero (African green monkey kidney) cells (Kumar *et al.*, 2001) and anti-neoplastic activity against Sarcoma.

Saundane *et al.* (2000) investigated the anti-inflammatory and analgesic activity of various extracts of *Leucas aspera*. Four different crude extracts- petroleum ether, chloroform, ethanol and water of *Leucas aspera* were investigated for anti-inflammatory and analgesic activities in albino rats and mice respectively at a dose of 400 mg/kg body weight, orally.

Bhalsing *et al.* (2001) reported that many plants in the Solanaceae family accumulate alkaloids based on a C27 cholestane skeleton, eg. Solasodine and tomatidine. These compounds are essentially nitrogen analogues of steroidal saponins, and they are usually present as glycosides, which have surface activity and hemolytic properties as do the saponins. Solanaceae family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance mainly steroidal lactones, glycosides, alkaloids and flavonoids.

Da Silva *et al.* (2003) studied the occurrence of flavones, flavonols and their glycosides in *Solanum* (Solanaceae). During the last decade several flavonoids of *Solanum* species have been isolated.

Amir and kumar (2004) have reported that various chemical constituents isolated from *Solanum* species, include alkaloids, phenolics, flavonoids, sterols, saponins and their glycosides. Alkaloids such as soladumlinidine and tomatidine were isolated from leaf and stem of *Solanum* species.

Mishra *et al.* (2005) showed that the leaves and bark extracts of *Ficus racemosa* possess anti-inflammatory activity (Rachel *et al.*, 2003). The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, laxative, diaphoretic and insecticide. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. In Nigeria, extracts or exudate of the plant are used as ear drops and in the treatment of boils, folklore and promoting wound healing (Igoli *et al.*, 2005).

Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman *et al.*, 2006 and Ostapowicz *et al.*, 2002). Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions (Ricaurte *et al.*, 2006 and Kanathur *et al.*, 2001). Simvastatin (Lipid Lowering Agent) competitively inhibits HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) in the formation of mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels.

The study by Shafiur Rahman *et al.* (2006) showed that *Leucas aspera* root may contain biologically active constituent(s) having CNS activity. Sukumar *et al.* (2007) investigated the RBC membrane stabilisation in an *in vitro* method by the drug isolated from *Leucas aspera*. The isolated compounds have been duly characterized by chromatographic and hydrolytic study as well as by UV spectral means.

There are four recognized components to the wound healing process such as inflammation, destruction, proliferation and maturation. Protease activity is a normal recognized part of this process (Walker and Bowler, 2007).

Solanum nigrum L. which is called as black nightshade is a common, short-lived perennial shrub. The leaf and fruits are used as traditional medicines with high nutraceutical, antiseptic, antidysentric and antidiuretic properties and it is also used for the treatment of many skin diseases, kidney disorders, non-communicable diseases and many other common ailments (Mohamed *et al.*, 2009 and Kavitha shree *et al.*, 2012).

Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids (Kalimuthu *et al.*, 2010). These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man.

Venkatachalam and Jebasan (2010) have shown that phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellents and ovipositor attractants. The secondary metabolites in different plants make up a vast repository of compounds with a wide range of biological activities.

A review of literature has revealed that plant metabolites such as alkaloids, flavonoids, glycosides, etc. play an important role in many activities including wound healing, cardio-tonic, analgesic, anti-inflammatory, anti-oxidant and antimicrobial activity (Noor Fathima *et al.*, 2011). Wounds can be described as any damage or break of the skin or underlying tissues. Medical treatment of wounds includes administration of drugs either locally or systematically in an attempt to aid wound repair (Savanth and Shah, 1998).

Hemendra *et al.* (2011) studied the purpose of the plants in genus *Leucas* that are widely used in traditional medicine to cure many diseases such as cough, cold, diarrhoea and inflammatory skin disorder. An overview of the phytochemical and pharmacological investigations on the *Leucas* species is presented in this review.

A study by Srinivasan *et al.* (2011) revealed that *Leucas aspera* is a source of medicinally active compounds and have various pharmacological effects. *Solanum nigrum* is a potential herbal alternative that acts as an anti-cancer agent (Jain *et al.*, 2011).

Subban Murugesan *et al.*, (2011) investigated preliminary phytochemical screening activity of various extracts of the leaves and revealed the presence of various classes of compounds such as amino acids, carbohydrates, flavonoids, gum, oil & resins, proteins, phenolic groups, saponins, steroids, tannins and terpenoids in *Memecylon umbellatum*.

Srinivasan (2011) found that the leaves of *Leucas aspera* are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.

An attempt has been made by Gogoi *et al.* (2012) to assess the constituents of *Solanum nigrum* L and *Solanum myriacanthus* Dunal from districts of Upper Assam and showed that the leaves of *Solanum nigrum* and *Solanum myriacanthus* possess phytochemical constituents that could account for the medicinal value of both species. These two plants could be used for treatment of various diseases such as cough, liver problem, stomach ache, skin disease, jaundice, tooth ache, etc.

Anand *et al.* (2012) carried out experiments to determine the possible chemical components from *Zehneria scabras* leaves by Gas Chromatography and Mass Spectrum.

The leaves of *S.nigrum* are used as poultice for rheumatic gouty joints, in skin diseases, as anti-tuberculosis and are said to produce diaphoresis (Jabar Zaman Khan Khattak *et al.*, 2012). Fresh juice of this herb is used for curing fever and alleviating pain.

Rajani Chauhan *et al.* (2012) reported that Black nightshade is the common name for it. Root, whole plant and leaves are used, but, fruits of black colour are not used as they possess toxicity, therefore they are not used for medicinal purposes. Reddish brown coloured fruits are used for edible purpose.

Ramya *et al.* (2012) in their study on two important medicinal plants, *Coleus aromaticus* and *Leucas aspera* have assessed the phytochemical components and antimicrobial activity. The organic solvents *viz.*, methanol and ethanol were used for the extraction.

Solanum nigrum L. is used in Indian traditional and folklore medicines to cure various ailments. Phytochemical constituents in different solvent extracts have been analysed. The composition of phytochemicals depends on the type of solvent system employed in extraction (Subramanian Ramya *et al.*, 2012).

Anandan *et al.* (2012) reported that *Leucas aspera* (Willd.) Linn. is a potential folklore medicinal plant used as an antipyretic and insecticide. In this study, fatty acid esters, fatty acid amide, triterpene, diterpene alcohols and phytochemicals were identified as the major chemical groups in the methanol fractions of *L. aspera* leaves.

Chavan *et al.* (2013) found that *Leucas cephalotes* and *Leucas aspera* plant is used traditionally in fever, cold, rheumatism, psoriasis and chronic skin eruptions. It has also been incorporated very frequently in number of Siddha and Unani preparations (Kirtikar and Basu, 1990).

Yashvanth *et al.* (2013) have detected various macro elements like carbon, oxygen, calcium, silica and aluminium in different parts of *Solanum nigrum*. Other elements found were iron, sodium, potassium, phosphorus and chlorine.

Uche *et al.* (2013) have studied the safety and phytochemical components of the aqueous leaf extract of *Leucas martinicensis*. The quantitative and qualitative phytochemical studies were conducted on the leaf extract. More than 50 medicinal plants were used in

folklore medicine and the modern researches have proved that the plants were having antibacterial property (Sivanantam *et al.*, 2013).

The extract of whole plant *Leucas aspera* were subjected to quantification of phytoconstituents such as total phenolic content and total antioxidant capacity by *in vitro* methods. Preliminary phytochemical screening of the extract (chloroform and aqueous ethanol) revealed the presence of various bioactive components which include alkaloids, flavonoids, steroids, cardiac glycosides, saponins and tannins (Latha *et al.*, 2013).

Rawani *et al.* (2013) have studied the biocontrol of aqueous and solvent extracts of mature leaves of *Solanum nigrum* L. and in qualitative phytochemical analysis reported the presence of some biochemical compounds such as, saponin, flavonoids, steroids and tannin.

Ananthan *et al.* (2014) have reviewed *Solanum nigrum* leaf powder and showed its methanol/water soluble fraction having strong antioxygenic activity in refined sunflower oil. On the other hand, ethyl acetate fraction exhibited marginal antioxygenic activity, whereas the water soluble fraction was practically devoid of any activity in refined sunflower oil. The result of this study confirmed the presence of antioxygenic compounds in leaves, in particular its methanol/water extracts showed great potential as a natural antioxidant to inhibit lipid peroxidation in foods.

Kadiyala Gopi *et al.* (2014) evaluated the anti-ophidian properties of ethnomedicinal herb *Leucas aspera* against Indian cobra, *Naja naja* venom enzymes. Methanolic extract of *Leucas aspera* was evaluated *in vitro* for its ability to inhibit the major enzyme activities of *Naja naja* venom including protease and hemolytic factors. The type of phytochemicals present in the extract was analyzed. The results obtained demonstrate for the first time that the methanolic extract of *Leucas aspera* possess anti-venom activity and could be considered as a potential source for the anti-ophidian metabolites.

The preliminary screening of leaves of *Leucas indica* showed the presence of alkaloids, flavonoids, carbohydrates, glycosides, steroids, saponins, fixed oils, protein and amino acids (Babu *et al.*, 2014).

Leaves of *Solanum nigrum* are used in folklore medicine to treat oral ulcers in India (Alkesh Patel *et al.*, 2014)

Phytochemical and pharmacological screening of medicinal plants and their extracts have revealed the presence of valuable compounds and provide insight into new ways of

treatment with new drugs. Phytochemical constituents are the basic source for the several pharmaceutical industries (Kala *et al.*, 2014).

The phytochemical analysis of *Solanum nigrum* showed the presence of alkaloids, terpenoids, flavonoids, saponins, steroids and phenols (Modilal *et al.*, 2015). *Leucas lanata* might be a potential source to treat many diseases (Dixit *et al.*, 2015)

Hawary *et al.* (2015) reported that the medicinal plants have been used for decades for the treatment of different diseases. *Solanum nigrum* has been used for the management of liver disorders, diabetes, infections, cancer, inflammations and peptic ulcer. The ethanolic extract of green fruits of *Solanum nigrum* revealed a marked cytotoxicity against breast and liver cancer cell lines. This activity is related to the high content of steroidal glycoalkaloids in *Solanum nigrum* L unripe green fruits.

CHAPTER III

MATERIALS AND METHOD

In the present study, fresh and dry leaf sample of two different medicinal plants were used. The medicinal plants were *Solanum nigrum* and *Leucas aspera*. A study was carried out on various biochemical and phytochemical aspects using different solvent extracts. **COLLECTION OF PLANT SAMPLES**

The fresh leaves of *Solanum nigrum* and *Leucas aspera* were obtained from Nelliyalam village in Nilgiri District of Tamil Nadu, India.

MORPHOLOGY OF THE PLANTS

SOLANUM NIGRUM

Systematic Position

Kingdom	-	Plantae
Class	-	Eudicots
Order	-	Solanales
Family	-	Solanaceae
Genus	-	<i>Solanum</i>
Species	-	<i>S. nigrum</i>

Description

- *Solanum nigrum* is a common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats (**Plate-1**).
- It reaches a height of 30 to 120 cm, leaves 4.0 to 7.5 cm long and 2 to 5 cm wide; ovate to heart-shaped, with wavy or large-toothed edges.
- The surfaces of leaves may be hairy or hairless, petiole 1 to 3 cm long with a winged upper portion.
- Erect stem.
- The flowers have petals greenish to whitish, recurved aged and surrounded by prominent bright yellow anther.

- Inflorescence is simple with extended cymes, 5 to 10 flowered.
- Calyces are 1.2 to 2.5 mm long, slightly deflexed to base of mature berry, sepals usually ovate.
- Corolla stellate, white with translucent basal star 5-7 mm radius, usually 1.5-3 times as long as calyx.
- Anther is yellow, 1.5-2.5 mm long. Pollens are 29.5-33.9 μ m diameter. Styles 2.8-3.5 mm long, not beyond anther.
- The berry is mostly 6 to 8 mm in diameter, dull black or purple-black. In India, another stain is found with berries that turn red on ripening. Seeds are 1.7 mm long 20-60 per berry.

Distribution

Solanum nigrum commonly known as black nightshade is a species of *Solanum* native of India.

Medicinal Uses

- *Solanum nigrum* leaves are used to treat mouth ulcers that happen during winter periods in Tamil Nadu.
- It is known as manathakkali keerai in Tamil Nadu and apart from its use as a home remedy for mouth ulcers, is used in cooling, like spinach.
- *Solanum nigrum* is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints and fever.
- The juice of the plant is used to treat ulcer and other skin diseases.
- The fruits are used as a tonic, laxative, appetite stimulant and for treating asthma and "excessive thirst".
- In North India, the boiled extracts of leaves and berries are used to treat liver-related ailments, including jaundice.
- In Assam, the juice from its roots is used against asthma and whooping cough.
- *S. nigrum* is a widely used plant that is considered to be antitumorogenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic and antipyretic.
- Berries possess tonic, diuretic and cathartic properties. Seeds are mostly useful in giddiness and thirstiness.
- The roots are used in ophthalmopathy, rhinopathy and hepatitis.

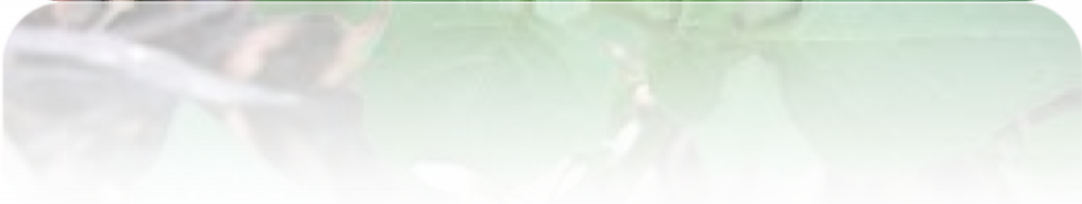


PLATE – 1
MORPHOLOGY OF THE PLANT *Solanum nigrum*

LEUCAS ASPERA

Systematic position

Kingdom	-	Plantae
Class	-	Eudicots
Order	-	Lamiales
Family	-	Lamiaceae
Genus	-	<i>Leucas</i>
Species	-	<i>L .aspera</i>

Description

- *Leucas aspera* is tropically found in dry, open, sandy soil and is abundant in areas with waste (**Plate-2**).
- *Leucas aspera* is an annual, branched herb, erect and the plant can reach heights of 15-60 cm.
- The leaves of *Leucas aspera* can be linear, lanceolate and pubescent up to 8.0 cm long and 1.25 cm broad.
- The length of the petiole is typically 2.5cm to 6 cm long.
- Flowers of *Leucas aspera* are white, small and directly attached to the base without a peduncular stalk. The flowers are in auxiliary whorls or dense terminals.
- The flowers possess 6mm long bracts that are bristle-tipped, linear and acute. They are "ciliate with long slender hair".
- The calyx is 8 to 13 mm in length and has a tubular shape. The bottom half is hispid and ribbed and small mouth and is very oblique. In addition, it also has small, short, triangular teeth.
- Corolla of *Leucas aspera* is 1 cm in length and the tube is 5 mm in length.
- The calyx is "densely white-woody", upper lip is approximately 3 mm in length and middle lobes rounded, obovate and the lateral lobes are sub-acute and small in size.
- Fruit of *Leucas aspera* is 2.5 mm long. They are nutlets that are brown, smooth and oblong in shape. The outer portion of the fruit is rounded, while, the inner portion is angular.

Distribution

Leucas aspera is commonly found throughout India and Philippines as well as the plains of Mauritius and Java.

Medicinal Uses

- *Leucas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities.
- It is also an antipyretic, it is an herb that has the ability to help reduce fevers.
- In addition, the juice of the flowers can be used to treat sinusitis, as well as headaches and also to treat intestinal worms in children.
- Flowers are valued as stimulant, expectorant, diaphoretic, insecticide and emmenagogue.
- Leaves are considered useful in chronic rheumatism, psoriasis and other skin eruptions. The leaves are applied mostly in snake bites.
- *Leucas aspera* is commonly used as an insecticide and in addition, the plant is also used in witchcraft.



PLATE - 2

MORPHOLOGY OF THE PLANT - *Leucas aspera*

BIOCHEMICAL PARAMETERS

The following biochemical parameters were observed in the leaves of the two medicinal plants taken for the present study.

- Chlorophyll
- Protein
- Carbohydrate

ESTIMATION OF CHLOROPHYLL CONTENT

Chlorophyll ' a', ' b ' and total chlorophyll were analysed following the method of Arnon (1949).

Materials Required

Analytical grade acetone was diluted to 80 % acetone.

Procedure

- One gram of freshly cut leaf sample was taken in a clean mortar.
- The leaf bits were ground to a fine pulp with the addition of 20ml of 80 % (w/v) acetone.
- The mixture thus obtained was centrifuged at 5000 rpm for 5 minutes.
- The supernatant was transferred to 100 ml volumetric flask. This procedure was repeated until the residue became colourless.
- The washing was collected and the volume was made upto the flask with acetone.
- The absorbance of the solution was read in a spectrophotometer at 645 and 663 nm against the solvent blank (80% acetone).

Calculation

The amount of chlorophyll present in the extract was calculated (mg chlorophyll/gm tissue) using the formula,

- mg chlorophyll 'a' / gm tissue = $12.7A_{663} - 2.69A_{645} \times \frac{V}{1000 \times W}$
- mg chlorophyll 'b' / gm tissue = $22.9A_{645} - 4.68A_{663} \times \frac{V}{1000 \times W}$
- mg total chlorophyll / gm tissue = $20.2A_{645} + 8.02A_{663} \times \frac{V}{1000 \times W}$

Where

A = Absorbance of specific wavelength

V = Final Volume of chlorophyll extract in 80% acetone

W = Fresh weight of tissue

ESTIMATION OF PROTEIN (Lowry *et al.*, 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin-Ciocalteu reagent by the amino acids, tyrosine and tryptophan present in the protein and the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured by Lowry's method.

Materials required

- Two percent sodium carbonate in 0.1 N Sodium hydroxide (Reagent A).
- 0.5 percent copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1 percent potassium sodium tartarate (Reagent B).
- Alkaline copper solution: Mix 50ml of reagent A and 1ml of reagent B prior to use (Reagent C).
- Folin-Ciocalteu reagent (Reagent D).
- Protein solution (stock standard): Accurately 50mg of bovine serum albumin was weighed and dissolved in water and made to up 50ml in a standard flask.

- Working standard: Ten ml of stock solution was diluted to 50ml with distilled water in a standard flask. One ml of this solution contains 200mg protein.

Procedure

Extraction of protein from samples

Extraction was carried out with buffers. About 500mg of the sample was weighed and ground well with a pestle and mortar in 5-10ml of the phosphate buffer, centrifuged and supernatant was used for protein estimation.

Estimation of Protein

- About 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard were pipetted out into a series of test tubes.
- About 0.1 and 0.2ml of the sample was pipetted out in two other test tubes.
- The volume was made up to 1ml using distilled water in all the test tubes. A tube with 1ml of buffer served as the blank.
- About 5ml of reagent C was added to each tube including the blank. Mixed well and allowed to stand for 10 minutes.
- To this, 0.5ml of reagent D was added, mixed well and incubated in dark for 30 minutes. Blue colour developed was read at 660nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in sample was expressed in mg / g

$$= \frac{\text{mg of protein}}{\text{Volume of test sample}} \times \text{concentration of the standard}$$

ESTIMATION OF CARBOHYDRATE CONTENT (Hedge and Hofreiter, 1962).

Anthrone Method

Principle

Concentrated sulphuric acid hydrolyses the glycoside bond of carbohydrate to the given monosaccharides which were then dehydrated to furfural. The furfural reacted with anthrone (10-Keto 9, 10- dihydroanthracene) to give the blue coloured complex which was measured calorimetrically at 630 nm.

Materials required

- 2.5 N HCl.
- Anthrone reagent was prepared by dissolving 200mg anthrone in 100ml of ice cold 95% H₂SO₄. Prepared freshly before use.
- Stock standard: 100mg of glucose was dissolved in 100ml water.
- Working standard: 5ml of stock standard solution was diluted to 100ml using distilled water (50mg/ml).

Procedure

- About 100mg of the sample was taken in a boiling tube and was hydrolysed by keeping it in boiling water bath for three hours with 5ml of 2.5N HCl and cooled at room temperature.
- Then it was neutralized with solid sodium carbonate until the effervescence ceases.
- The volume was made up to 100ml and centrifuged.
- The supernatant was collected and 0.5 and 1ml aliquot were taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard and a blank was maintained.
- The volume was made up to 1ml in all the tube including the sample tube by adding distilled water.
- Then, 4ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- Then, it was cooled rapidly and blue green colour developed was read at 630nm.
- A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrate present in the sample was calculated.

Calculation

The amount of carbohydrate present in 100mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

STATISTICAL ANALYSIS

The data obtained from various biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978).

PREPARATION OF LEAF POWDER

The leaves of both the medicinal plants taken for the present study were collected, cleaned and air dried, under shade for about three weeks. After drying, the leaves were then powdered using a mixer. This fine powder was analysed for phytochemical study (Plate 3 & 4).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The leaf powders of both the plants were dissolved in various solvents and the preliminary phytochemical tests were carried out using Harborne (1984).

Test for Alkaloids

Mayer's Reagent

To 1ml of the extract, 2ml of Mayer's reagent was added. Appearance of dull white precipitate indicated the presence of alkaloids.

Test for Flavonoids

To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.



PLATE - 3

Leaf powder of the *Solanum nigrum*



PLATE - 4

Leaf powder of the *Leucas aspera*

Test for steroids

Lieberman-Burchard's Test

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Test for Terpenoids

Salkowski Test

5ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of terpenoids.

Test for Quinone

To 1ml of extract, a few drops of concentrated hydrochloric acid was added. A yellowish brown colour observed, showed the presence of quinone.

Test for Phenols

To 1ml of extract, lead acetate solution was added and the precipitate formation indicated the presence of phenolic compounds.

Test for starch

To 1ml of extract, a few drops of iodine solution was added. Any characteristic colour change showed the presence of starch.

Test for Anthocyanin

NaOH Test

A small amount of extract was treated with 2ml of NaOH and observed for the formation of blue green colour.

Test for Proteins

Ninhydrin Test (Acetone)

Ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed for the formation of purple colour.

Test for carbohydrates

Molisch's Test

2 drops of Molisch reagent was added to an aqueous or hydrochloric acid solution of the extract and 2ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for Cellulose

To 1ml of extract, a few drops of iodine solution was added followed by a few drops of sulphuric acid. Dark brown (or) red colour observed, showed the presence of cellulose.

Test for Fixed Oil and Fat

To 1ml extract, a few drops of Sudan III solution was added. A shining orange colour obtained showed the presence of fixed oil and fat.

CHAPTER – IV

RESULTS AND DISCUSSION

The present investigation of the biochemical parameters and phytochemical constituents of the leaf extracts of the two medicinal plants, *Solanum nigrum* and *Leucas aspera* showed the following results.

BIOCHEMICAL ANALYSIS OF THE LEAVES OF *Solanum nigrum* and *Leucas aspera*

The biochemical parameters were analysed using fresh leaf samples.

ESTIMATION OF CHLOROPHYLL "a", CHLOROPHYLL "b" AND TOTAL CHLOROPHYLL CONTENT

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content was calculated for the medicinal plants.

The chlorophyll 'a' content of *S. nigrum* and *L. aspera* was estimated to be 0.21 ± 0.01 mg and 0.477 ± 0.15 mg respectively (Table-1 and Figure-1).

The chlorophyll 'b' content was estimated to be minimum in *L. aspera* (0.023 ± 0.16 mg), when compared to *S. nigrum* (0.076 ± 0.03 mg).

The total chlorophyll content was higher in *L. aspera* and the value was 0.450 ± 0.04 mg (Table-1, Figure-1). *S. nigrum* showed a total chlorophyll content of 0.275 ± 0.04 mg (Table-1, Figure-1).

Faisal and Anis (2006) have reported higher amount of chlorophyll a (0.91 ± 0.19 mg/g FW) and chlorophyll b (0.61 ± 0.09 mg/g FW) in plants of *Psoralea corylifolia* compared to chlorophyll a (0.83 ± 0.31 mg/g FW) and chlorophyll b (0.53 ± 0.14 mg/g FW) in seedlings. The amount of chlorophyll a and chlorophyll b in normal leaf was less when compared to the regenerated leaf.

The increased *S. nigrum* content of chlorophyll in *S. nigrum* may increase the efficiency of photosynthesis by the plants (Salisbury, 1991). The lowest pigment levels were observed in plants treated with 100mM NaCl. The salt stress leads to stomatal closure, which

reduces CO₂ consumption in leaves and suppress carbon fixation and lead to reduction in photosynthetic rate (Karthikumar *et al.*, 2007).

Table- 1

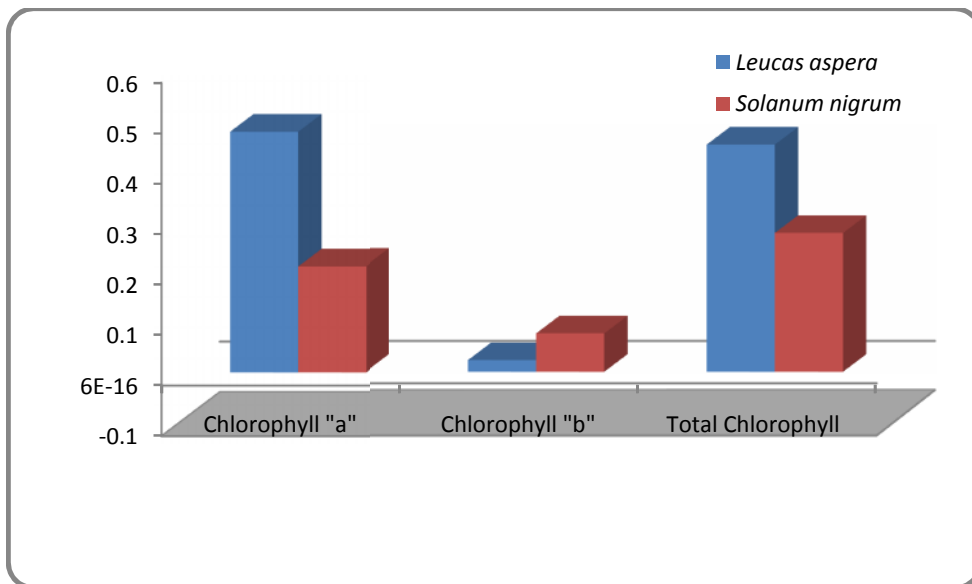
Chlorophyll ‘a’, Chlorophyll ‘b’ and Total Chlorophyll content of the two medicinal plants

Chlorophyll content (mg/g of leaf sample)	Medicinal plants	
	<i>Leucas aspera</i>	<i>Solanum nigrum</i>
Chlorophyll ‘a’	0.477 ± 0.15	0.21 ± 0.01
Chlorophyll ‘b’	0.023 ± 0.16	0.076 ± 0.03
Total Chlorophyll	0.450 ± 0.04	0.275 ± 0.04

Values are mean ± SD triplicates

Figure-1

Chlorophyll ‘a’, chlorophyll ‘b’ and Total chlorophyll content of the two medicinal plants



ESTIMATION OF PROTEIN

The protein estimated in the two medicinal plants taken for the study are presented in Table -2 and Figure -2. The protein content was found to be 7.4 ± 3.11 mg in *S. nigrum* and 10.8 ± 1.70 mg in *L. aspera* in 0.1ml of the extract.

Recent investigations on medicinal plants used in traditional medicine have led to the discovery of many new drugs and hundreds of pharmacologically active substances for synthetic modifications (Wang, 2008).

Nupo *et al.* (2013) have studied the effect of squeezing, blanching and drying on the nutritional value of leafy vegetables such as *S. nigrum*. The result of the analysis of the samples showed that the protein content of the dried sample had the highest vitamin A, E and D content. This study indicates that *S. nigrum* is a good source of diet especially when processed.

Protein content in *S. nigrum* increased significantly with increase in NaCl concentration (Osman *et al.*, 2007). The concentration of protein showed gradual increase from 35% to 90% (100mM). Proteins that increase in plants saline conditions may provide storage form of nitrogen that is re-utilized later (Singh *et al.*, 1987) and may play a role in osmotic improvements.

Table - 2

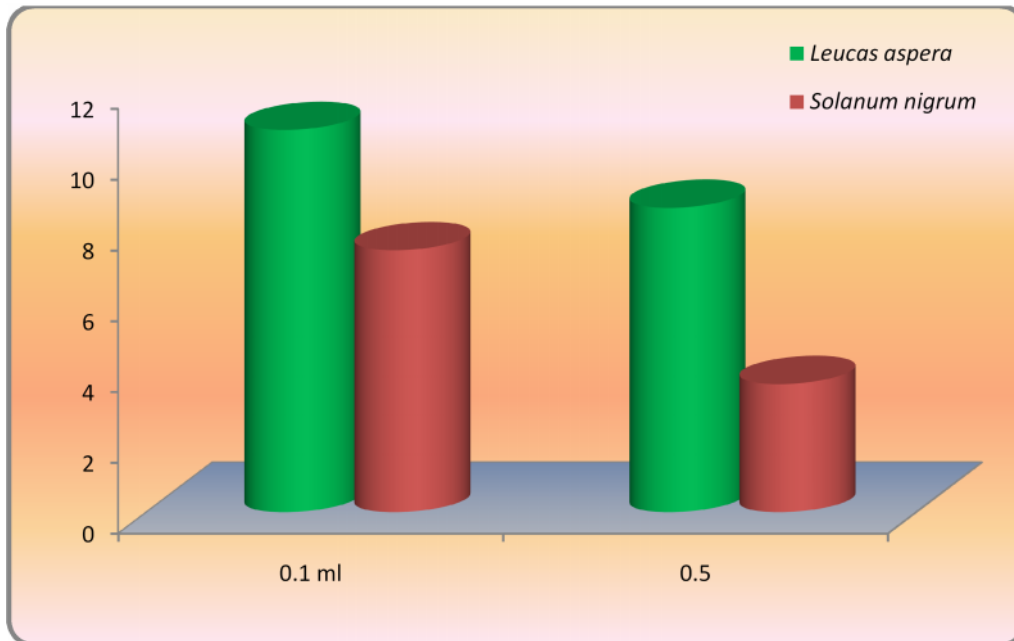
Protein content of the two medicinal plants

Plant	Protein (mg / gm of leaf sample)	
	0.1 ml	0.5 ml
<i>Leucas aspera</i>	10.8 ± 1.70	8.6 ± 0.51
<i>Solanum nigrum</i>	7.4 ± 3.11	3.62 ± 0.54

Values are mean ± SD of triplicates

Figure - 2

Protein content of the two medicinal plants



ESTIMATION OF CARBOHYDRATE

The carbohydrate content was estimated to be 1.57 ± 0.19 mg in *S. nigrum* and 1.50 ± 0.20 mg in *L. aspera* in 0.2 ml concentration. In 0.1ml of the extract, the values were found to be almost similar (1.4 ± 0.21 and 1.4 ± 0.14 in *L. aspera* and *S. nigrum* respectively) (Table - 3 and Figure - 3).

The ash content of the leaves is similar to the values reported for some commonly known leafy vegetables in Nigeria, including *Ocimum gratissimum*, *Abelmoscetus esculentus* and *Ipomoea batata*. It is however, lower than the reported value of the *Talinum triangulare* (Akindahunsi and salawu, 2005; Antia, 2006).

The oil contains many essential fatty acids, and has potential nutritional value and this can be used as nutraceuticals. The presence of unsaturated fatty acids, namely, linoleic and oleic acids reflects considerably on the nutritional value.

The use of nutraceuticals as attempts to accomplish desirable therapeutic outcomes with reduced side effects, as compared with other therapeutic agents has met with great success. So, we may utilize *S. nigrum* for the processing of nutraceuticals by way of giving proper resource management. The wealth of this weed can be capitalised for the benefit of human beings. However, eco-friendly utilization of this species is also possible in those countries where this weed already exists. The pharmaceutical and biotechnological companies should hold this with great commitments and need to address it in the urgency it deserves (Hemen Sarma, 2011).

Carbohydrates are the primary source of energy for the body and are often referred to as 'fuel of life'. Each gram of carbohydrate yields 4 calories in the process of its metabolism. They help to provide energy for muscular work and nutritive processes, maintenance of body temperature, besides their role in oxidation of fats, and as spare protein for growth and repair. Therefore, daily consumption of such a leafy vegetable can significantly contribute to the recommended daily intake of total carbohydrate, which is about 130 g (Chau, 1997).

Table - 3

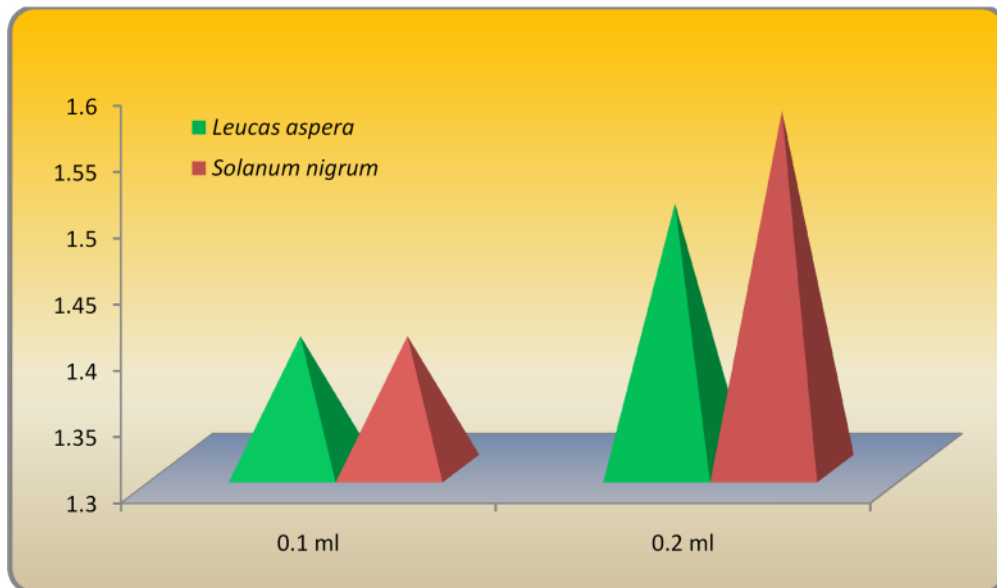
Carbohydrate content of the two medicinal plants

Plant	Carbohydrate (mg / gm of leaf sample)	
	0.1 ml	0.2 ml
<i>Leucas aspera</i>	1.4 ± 0.21	1.50 ± 0.20
<i>Solanum nigrum</i>	1.4 ± 0.14	1.57 ± 0.19

Values are mean ± SD of triplicates

Figure - 3

Carbohydrate content of the two medicinal plants



PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAVES OF *Leucas aspera* and *Solanum nigrum*

In the present study, a preliminary phytochemical analysis was carried out using dry leaf powder to identify the active constituents such as, alkaloids, flavonoids, steroids, terpenoids, quinone, oil and fat, phenols, starch, anthocyanin, protein, carbohydrate and cellulose present in the leaves of both the medicinal plants.

The dried and powdered leaves of *L. aspera* and *S. nigrum* were extracted using various solvents viz., water, ethanol, chloroform, petroleum ether and benzene and the extracts thus obtained were analysed for the presence or absence of secondary metabolites.

The phytochemical screening of *S. nigrum* leaves showed the presence of alkaloids, flavonoids, steroids, terpenoids, quinone, phenols, starch, cellulose, oil and fat in all the five extract used (Table-4).

Anthocyanin was absent only in water and chloroform extracts. Protein and carbohydrate were present only in water and ethanol extracts (Table-4).

Dried powder of the whole plant of *Stevia rebaudiana* was extracted with n-hexane, ethyl acetate and ethanol which were subjected to various chemical tests to ascertain the main constituents of the plant (Das *et al.*, 2011). The results revealed the presence of significant amounts of alkaloids, glycosides, tannins and flavonoids in ethanol extract, while, the other two extracts contain moderate amount of the chemical constituents.

Sridhar *et al.* (2011) have shown that *Solanum nigrum* L. is an important herbaceous medicinal plant. The herb is antiseptic, antidysentric, antidiueretic and is recommended in Ayurveda for the management of gastric ulcers. Phytochemical screening of the crude extracts revealed the presence of secondary compounds such as alkaloids, flavonoids, steroids, tannins and phenols.

The phytochemical screening of leaf of *L. aspera* showed the presence of various phytoconstituents. Further studies are still necessary to clear up a mechanized way how the plant contributes in cytotoxic and other pharmacological properties (Nipa chowdhury, 2012).

The dry leaf powder extracts of *L. aspera* showed the presence of alkaloids, flavonoids, steroids, terpenoids, quinone, phenols, starch, cellulose, oil and fat in all the solvent extracts. Protein and carbohydrates were present in aqueous and ethanol extract (Table-5).

Table - 4
Qualitative analysis of the phytochemical constituents of *Solanum nigrum*
in various solvents extracts

S.NO	Name of the test	Water	Ethanol	Chloroform	Petroleum ether	Benzene
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Steroids	+	+	+	+	+
4	Terpenoids	+	+	+	+	+
5	Quinone	+	+	+	+	+
6	Phenols	+	+	+	+	+
7	Starch	+	+	+	+	+
8	Anthocyanin	-	+	-	+	+
9	Protein	+	+	-	-	-
10	Carbohydrats	+	+	-	-	-
11	Cellulose	+	+	+	+	+
12	Oil & Fat	+	+	+	+	+

‘+’ - Present ‘-’ - Absent

Anthocyanin was present in four of the five extracts except chloroform (Table-5). Protein and carbohydrates were present in aqueous and ethanol extract.

Ramalingam *et al.* (2012) have shown the presence of alkaloids, tannins, saponins, flavonoids, phenolic compounds and glycosides in *Leucas aspera*.

Chen (2009) has carried out analysis in *S.nigrum* fruit and has reported the presence of two novel disaccharides along with protein, fibre, carbohydrate and minerals like magnesium, phosphorus and vitamin C, B and folic acid.

Alkaloids such as soladunalinidine, solasonine and solamargine have been isolated from the leaf of *Solanum* species (Juneja *et al.*, 2007).

The presence of the above said phytochemical constituents could account for the much medicinal properties of both the species for the treatment of various diseases/ailments such as cough, liver problem, stomach-ache, skin diseases, inflammation, jaundice, tooth ache etc which have been reported by a number of workers (Ravi, 2009).

Table - 5
Qualitative analysis of the phytochemical constituents of *Leucas aspera*
in various solvent extracts

S.NO	Name of the test	Water	Ethanol	Chloroform	Petroleum ether	Benzene
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Steroids	+	+	+	+	+
4	Terpenoids	+	+	+	+	+
5	Quinone	+	+	+	+	+
6	Phenols	+	+	+	+	+
7	Starch	+	+	+	+	+
8	Anthocyanin	+	-	-	+	+
9	Protein	+	+	-	-	-
10	Carbohydrats	+	+	-	-	-
11	Cellulose	+	+	+	+	+
12	Oil \$ Fat	+	+	+	+	+

‘+’ - Present ‘-’ – Absent

The present study on *S. nigrum* and *L. aspera* has shown the presence significant phytochemical constituents. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more phytochemical drugs of natural origin.

Plant steroids are known to be important for their cardiogenic activities, insecticidal and anti- microbial properties. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory (Abubakar, 2009) properties.

Senthilkumar and Reetha (2009) have reported that methonal extract of *Aegle marmelos* and *Cassia auriculata* showed higher antimicrobial, fungicidal, immunostimulant, antibacterial, antiviral, analgesic, anti-inflammatory, antitumor, cytotoxic, anthelmintic, expectorant and antitussive activities.

Chandrashekar (2013) have worked with leaf of *L. indica* and showed the presence of glycosides in water and ethanol. The other solvents such as chloroform, acetone and petroleum ether showed the presence of steroids only.

CHAPTER-V

SUMMARY AND CONCLUSION

The present investigation has revealed the presence of various biochemical compounds such as chlorophyll, protein and carbohydrate contents in both the medicinal plants (*Solanum nigrum* and *Leucas aspera*) taken for the study.

Among the two medicinal plants studied, the protein, chlorophyll 'a' and total chlorophyll were significantly higher in *L. aspera*. The carbohydrate and chlorophyll 'b' content was found to be higher in *S. nigrum*.

The preliminary phytochemical screening showed the presence of almost all the phytoconstituents in the different solvent extracts used. Protein and carbohydrate were found to be absent only in chloroform, petroleum ether and benzene extracts.

The presence of various other phytoconstituents such as alkaloids, flavonoids, sterols, terpenoids, quinone, phenols, starch, cellulose, oil and fat shows the therapeutic use of the plants both *S. nigrum* as well as *L. aspera* for accelerating the wound healing process.

The leaf extracts of the plants may serve as a potential tool that could be used in mosquito control. The phytoconstituents may be isolated and used for treating cold, cough, headache and also to treat intestinal worms in children. The leafy vegetables can be used to treat chronic rheumatism.

The naturally occurring phenolic compound in the two medicinal plants could be considered as a potential remedy for breast and liver cancer. In lieu of the nutrient and chemical constituents present in the two medicinal plants namely *S. nigrum* and *L. aspera* both the plants could be supported as a safe and highly important medicinal plants for mankind.

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